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ONE-STEP CHROMATOGRAPHIC CLEANUP OF CHLORINATED HYDROCARBON  
PESTICIDE RESIDUES IN BUTTERFAT. II. CHROMATOGRAPHY ON FLORISIL

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chlorinated hydrocarbons  
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## One-Step Chromatographic Cleanup of Chlorinated Hydrocarbon Pesticide Residues in Butterfat. II. Chromatography on Florisil

By WILLIAM A. MOATS (U.S. Department of Agriculture, Agricultural Marketing Service, Market Quality Research Division, Beltsville, Md.)

**The procedure for chromatography on Florisil was modified by using methylene chloride-petroleum ether mixtures to improve separation of insecticides from fat. Combined with paper chromatography, it provides a screening procedure for insecticide residues in dairy products that is comparable in speed and simplicity to total chloride methods but is of superior sensitivity.**

A previous paper (1) described the use of silicic acid for one-step chromatographic cleanup of some chlorinated hydrocarbon insecticides in butterfat. The silicic acid procedures, however, could be used with only a limited number of insecticides; they are not suitable as general screening tests. A number of other common adsorbents were, therefore, studied to develop a more generally applicable procedure. Florisil has proved to be the most generally satisfactory adsorbent for this purpose.

Florisil was used by Ordas, *et al.* (2) for chromatographic cleanup of heptachlor residues in alfalfa. Florisil columns were later used by Mills (3) and by McKinley and Mahon (4) as a cleanup step following acetonitrile partition in general screening procedures. Mills (3) reported difficulty in eluting kelthane and the aldrin group of insecticides from Florisil and had to use other types of columns for their cleanup. Mills (5) reported difficulty in obtaining good enough cleanup to permit spotting the entire sample for paper chromatography by Mitchell's procedure (3, 6).

In the present method, several modifications were made in the procedure for chromatography on Florisil to enhance its usefulness. The use of methylene chloride-petroleum ether mixtures, instead of 6:94 ethyl ether-petroleum ether used by other authors (3, 4, 7) for elution, improves sepa-

ration of insecticide from fat enough to permit direct chromatographic cleanup for paper chromatography of up to 2 g of butterfat. When the Florisil was deactivated slightly by adding water, it was possible to recover the aldrin group of insecticides satisfactorily.

This simple and rapid cleanup procedure, together with the paper chromatographic techniques of Mitchell (3, 6), provides a usable screening procedure for most common chlorinated insecticides that compares in speed and simplicity to total chloride methods (8) and at the same time is more sensitive and a good deal more informative.

### METHODS

#### *Reagents and Equipment*

*Petroleum ether*.—30–60°, redistilled.

*Methylene chloride*.—Reagent grade, redistilled.

*Florisil*.—60–100 mesh, activated at 1200°F (Floridin Company, Tallahassee, Fla.), standardized as described below.

*Sodium sulfate*.—Anhydrous, reagent grade, granular.

Butterfat used was obtained from commercial sources. E.S.A. standard insecticides were used except as indicated.

Equipment required is as described previously for chromatography in silicic acid (1). All stopcocks should be Teflon.

#### *Procedure*

*Standardization of Florisil*.—Heat Florisil overnight in an oven at 130–150°C, transfer to tared screw-top jars, cool, and weigh. Add 5 ml water/100 g Florisil, shake thoroughly, and allow to stand a day or two.

Check Florisil activity as follows: Fortify 0.5 g butterfat in 10–20 ml petroleum ether with a few  $\mu$ g of DDT and methoxychlor. Prepare column of 25 g Florisil (about 5") in a 20  $\times$  400 mm chromatographic tube with coarse fritted glass disk, and top with about 1" anhydrous sodium sulfate. Wash with 50 ml methylene chloride and 50 ml petroleum

ether. Discard washings and put receiving flask in place. Add sample to column and, as soon as it has sunk in, rinse sample flask and inside of chromatographic tube with 10–20 ml petroleum ether and add this to column. Elute with 75 ml 20% v/v methylene chloride in petroleum ether (fraction 1). Change receivers and elute with an additional 175 ml 20% methylene chloride (fraction 2), collecting fractions in Erlenmeyer flasks of appropriate size.

To concentrate, add small boiling chip to sample flask, and boil in steam bath until only a few ml of liquid remains. (Boiling to dryness is not harmful, although prolonged heating of dry residue might cause some loss of insecticides.) Let flask cool, and transfer contents to a 15 ml conical centrifuge tube. Rinse flask with few ml of petroleum ether and add to centrifuge tube. (Total volume in tube should not exceed 10 ml.) Add very small boiling chip. Place tip of tube in steam bath and adjust steam so that contents boil vigorously but without mechanical loss. Boil just to dryness and let tube cool. Use 0.1–0.2 ml liquid that condenses in bottom to spot for paper chromatography. To concentrate further, let tube stand open an hour or two. Use of an air stream at any stage in the concentration may cause serious loss of insecticides.

Run paper chromatograms, using Mitchell's nonaqueous system with dimethylformamide as immobile solvent and trimethylpentane as mobile solvent (3, 6). If insecticide spots tend to move with solvent front, as is frequently the case in the summer because of higher humidity, increase dimethylformamide concentration in dipping tank from 35% to 45 or 50%. Spray and develop chromatograms as described by Eiduson (9). Let papers stand after spraying until they appear thoroughly dry before exposing to UV light. DDT should be all in fraction 1, methoxychlor in fraction 2. Fraction 1 should give a light ring of fat when it is spotted; fraction 2 should show appreciably less fat. If these conditions are not met, repeat as above, using 15% methylene chloride to elute column.

Each new lot of Florisil received from the manufacturer should be standardized. It was not necessary to restandardize every fresh batch of Florisil prepared from a given lot. The material is stable for at least a month after processing as described.

#### **Preparation of Unknown Samples**

*Butter.*—Melt butter in beaker and decant

fat layer. Dissolve 2 g of the anhydrous butterfat in 30–40 ml petroleum ether and proceed directly with chromatography.

*Milk.*—Extract as described by Mills (3) but in a separatory funnel. (Layers normally separate satisfactorily without centrifugation.) Evaporate or boil to dryness, take up residue in petroleum ether, and proceed with chromatography, using an aliquot equivalent to 50 ml original milk.

#### **Chromatography**

Pack 100 g Florisil into 40 × 600 mm chromatographic tube and top with 1" anhydrous sodium sulfate. Proceed as described under *Standardization*, multiplying all amounts by 4. For elution, use methylene chloride concentration (15 or 20%) found to be satisfactory when Florisil was standardized.

#### **Recovery Experiments**

The procedure for unknowns was followed with 2 g butterfat fortified with 2–5  $\mu$ g of the insecticide. Recoveries were estimated by comparison with standard amounts of insecticide on the paper chromatograms. Several knowns were run at the same time where  $R_f$  values permitted.

#### **Discussion**

The purpose of this study was to develop a one-step chromatographic cleanup technique suitable for separating a wide range of chlorinated hydrocarbon insecticides from fairly large amounts of fat. This together with paper chromatography would provide a fairly simple and rapid screening procedure. Paper chromatography requires quite rigorous cleanup for satisfactory results. Adsorbents examined with this idea in mind were silicic acid, activated alumina, decolorizing carbons, Florisil, and fuller's earths. The results with silicic acid have been reported previously (1). No worthwhile results were obtained with alumina. The last three adsorbents showed some promise, with Florisil being the most satisfactory.

Decolorizing carbons would also separate a large number of common insecticides from butterfat. Separation from fats was not as efficient as with Florisil, however, and this fact limited sample size. Some interfering material was washed off the carbon during elution. Despite these difficulties, decolorizing carbon may be useful for cleanup of

such insecticides as Thiodan which cannot be completely recovered from other types of adsorbents.

Several commercial types of fuller's earth gave results rather similar to those obtained with Florisil, but the separations from fat were not as satisfactory.

In preliminary experiments, some lots of Florisil which had been on the shelf for over a year were used without reactivation. Elution with 20% methylene chloride in petroleum ether gave the most satisfactory separations of insecticides from fat (see Table 1). Methoxychlor was the most difficult to elute of the common insecticides. Dieldrin and endrin were eluted in somewhat less volume than methoxychlor. When the work was repeated with fresh and more active Florisil, the earlier results could be duplicated by increasing the concentration of methylene chloride in the eluting solvent to 40%, except that dieldrin and endrin could not be recovered (Table 2). When Florisil was deactivated by adding 5 ml of water per 100 g of Florisil, the earlier results could be reproduced. Mills (3), using carefully activated Florisil, also reported difficulty in recovering dieldrin and endrin. Johnson (7), using activated Florisil, found that a much higher concentration of ethyl ether was required to elute dieldrin and endrin than other chlorinated hydrocarbon insecticides and that separations from fat were not sufficient for paper chromatography.

Mills (3) also reported difficulty in eluting kelthane and aldrin from Florisil, although in the present work, they were recovered from even the more active lots without difficulty. He also reported poor recoveries of lindane, probably due to loss during evaporating samples under an air stream rather than failure to elute the material from Florisil.

The results of several determinations with the procedure adopted are shown in Table 3. Most recoveries were nearly quantitative. Only one isomer of Thiodan was recovered. Ovex (*p*-chlorophenyl-*p*-chlorobenzenesulfonate) could also be recovered from Florisil but somewhat more than 1000 ml of elutant was usually required. Thiodan alcohol and

**Table 1. Chromatography on aged Florisil (80 g column, 2 g butterfat, 2-5  $\mu$ g insecticides; eluting agent: 20% v/v  $\text{CH}_2\text{Cl}_2$  in petroleum ether)**

Insecticide	Estimated Recoveries* of Insecticides by Paper Chromatography from Indicated Fraction, %			
	0-200 ml	200-400 ml	400-600 ml	600-800 ml
Aldrin	90-100	0	0	0
Chlordane	100	0	0	0
Lindane	100	0	0	0
DDE	100			
DDT	100	0	0	0
Endrin	0	70	30	0
Heptachlor	100	0	0	0
Heptachlor epoxide	40	60	0	0
Ovex	0	0	50	50
Toxaphene	100	0	0	0
Kelthane	20	80	0	0
Methoxychlor	0	40	60	Tr.
Dieldrin	0	60	40	0

\* Estimated to  $\pm 10\%$ .

**Table 2. Chromatography on new Florisil (80 g column, 2 g butterfat, 2-5  $\mu$ g insecticide; eluting agent: 40% v/v methylene chloride in petroleum ether)**

Insecticide	Estimated Recoveries* of Insecticides by Paper Chromatography from Indicated Fraction, %	
	0-600 ml	600-800 ml
Aldrin	100	0
Dieldrin	0	0
Endrin	Tr.	0
DDT	100	0
DDE	100	0
Methoxychlor	100	Tr.
Lindane	100	0
Heptachlor epoxide	100	0
Kelthane	100	0

\* Estimated to  $\pm 10\%$ .

2,4-D acid could not be eluted from Florisil.

All non-oxygenated chlorinated hydrocarbon insecticides were eluted completely in the first 300 ml. Oxygenated compounds required a somewhat larger volume. By



**Table 3. Chromatography on Florisil heated and deactivated with 5 ml water/100 g Florisil**  
**(100 g column, 2 g butterfat, 2-5  $\mu$ g insecticides;**  
**eluting agent: 15 or 20%  $\text{CH}_2\text{Cl}_2$  in petroleum ether)**

Insecticide	No. of Determinations	Estimated Recoveries <sup>a</sup> of Insecticides by Paper Chromatography from Indicated Fraction, %			
		0-300 ml	300-600 ml	600-1000 ml	Total Recovery
Aldrin	2	100	0	0	100
Chlordane	1	100	0	—	100
DDE	2	100	0	0	100
DDT	3	100	0	0	100
Dieldrin	4	0	50-90	10-50	100
Endrin	1	0	—100—	—	100
Heptachlor	2	100	0	—	100
Heptachlor epoxide	3	40	60	0	100
Kelthane	4	30-50	50-70	0	100
Lindane	4	100	0-Tr.	0	100
Methoxychlor	3	0	30	70	100
Thiodan	1	0	—100—	—	100
Toxaphene	1	100	—0—	—	100
DDD	2	100	—0—	—	100
Ovex	3	0	—50-100—	—	50-100

<sup>a</sup> Estimated to  $\pm 10\%$ .

<sup>b</sup> One component only.

collecting the eluate in two fractions, dieldrin, endrin, methoxychlor, and the one isomer of Thiodan were separated completely from the non-oxygenated insecticides. Heptachlor epoxide and kelthane were split between the 0-300 and 300-1000 ml fractions.

When butterfat was added, a characteristic blue band appeared at the top of the column, evidently produced from interaction of carotenes with Florisil. A similar reaction has been reported with carotenes and fuller's earth (10). The band spread out and faded during elution.

Because Florisil permits high flow rates through the column, this is a rapid procedure even though rather large volumes of solvent are used. Reducing the flow rate did not improve or alter the results; thus it is advantageous to use the maximum possible rate of elution. The cleanup, from preparation of the column to the time the sample is ready for paper chromatography, nor-

mally required about 90 minutes, half of which was spent concentrating the eluate.

These results show the importance of steric factors in adsorption of some compounds. From the usual considerations of polarity and functional groups, one would not expect dieldrin and endrin to be adsorbed much more strongly than heptachlor epoxide, which also is a chlorinated compound with an epoxide grouping. This is the case with the less active Florisil. However, with the highly active material, dieldrin and endrin are much more strongly adsorbed than even methoxychlor, indicating that some peculiarity in the surface of the highly activated Florisil gives it a special affinity for dieldrin and endrin. Thiodan shows two components on paper chromatography, presumably isomers, only one of which can be recovered from Florisil. In earlier work, it was similarly observed that lindane was not readily eluted from silicic acid, although it was easily eluted

from silicic acid-sulfuric acid mixtures and from Florisil. Again, it is necessary to refer to steric factors to explain these results.

For paper chromatography of samples cleaned up by this method, Mitchell's non-aqueous system using dimethylformamide and trimethylpentane (3, 6) was distinctly the most satisfactory of several tested. With this system, interfering materials moved with the solvent front, and fat in the sample had little or no effect on  $R_f$  values. With the aqueous system, troublesome washing of papers is required and movement of insecticide spots in samples is sometimes retarded relative to standard spots containing no fat, interfering with identification of unknowns. Compounds can usually be identified satisfactorily from paper chromatograms using the nonaqueous system and from their location in the eluate from the columns.

In summer, insecticide spots may move with the solvent front on paper chromatograms, probably because they absorb moisture from the air (11). This difficulty is readily overcome by increasing the dimethylformamide concentration in the dipping solvent to 45 or 50%, or even higher, if necessary.

The technique for spraying and developing the insecticide spots is quite important if maximum sensitivity is to be obtained. All insecticides studied except toxaphene and perthane are readily detected at a level of 1  $\mu$ g on paper chromatograms and in many cases, a few tenths of a microgram may be detected. Toxaphene forms a streak and requires 2-3  $\mu$ g to show up, while 5  $\mu$ g of perthane gives a barely visible spot. For a 2 g fat sample, then, 0.5 ppm or less of most insecticides can be detected, and this should be adequate for screening purposes. For greater sensitivity a fat sample of suitable size can be carried through a preliminary acetonitrile partition as described by Mills (3) or by McKinley and Mahon (4) and then a final cleanup made on Florisil as described in the present paper, using a column of appropriate size.

### Conclusion

Cleanup on Florisil columns followed by paper chromatography provides a rapid and broadly applicable screening method for insecticide residues in dairy products. The apparatus required is simple and inexpensive. This cleanup technique can probably be adapted for detection of residues in other food and agricultural products.

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